HISTOGENESIS OF THE PROVENTRICULAR SUBMUCOSAL GLAND OF THE CHICK AS REVEALED BY LIGHT AND ELECTRON MICROSCOPY

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ABSTRACT

The development of the submucosal tubuloalveolar glands of the proventriculus was studied with both light and electron microscopes, with special reference given to the 13–18-day period. Beginning as an invagination in the stratified columnar epithelium lining the lumen of the proventriculus, the simple tubular gland, by repeated bifurcation of the invaginated portion, by sloughing of the superficial cells, and by remodeling of the residual basal cells from columnar to cuboidal, becomes a compound tubuloalveolar gland composed of numerous secretory units. Concomitant with the gross cellular changes, ultrastructural changes in the intercellular membranes are evident. By day 4 after hatching, the cells forming the secretory units appear to be functional.

INTRODUCTION

The avian stomach is peculiar in that it consists of two morphologically and physiologically distinct parts: the glandular portion, or proventriculus, and the muscular portion, or gizzard. The proventriculus is a relatively thick-walled structure located at the distal end of the oesophagus and containing both simple mucous-secreting glands and compound submucosal tubuloalveolar glands. The gizzard is a thick muscular bulb, the mucosa of which contains simple glandular cells which secrete a tough, horny layer.

The proventriculus has been described by Cazin (1886, 1887), Zietschmann (1908), Calhoun (1933, 1954), Batt (1924), and Bradley and Graham (1950). Aitken (1958) and Toner (1963) further amplified the literature with specific reference to the compound submucosal glands.

Sjögren (1941) and Hibbard (1942) described the embryonic development of the submucosal glands. The former, having studied material ranging from 3 days through 15 days of incubation, reported that the glandular primordia begin at day 7, developing into about 65 invaginations. By day 14 the 8-vesicular stage is reached. The glandular epithelium changes from cylindrical to cuboidal type as the glands become multilobular. According to Hibbard, the invaginating glands are prominent at day 7 and considerably deeper by day 9. The simple pockets begin to branch on day 10, with branching becoming increasingly complex through day 13. By day 14, glandular branching is much greater and the rapidly dividing cells are smaller. The glandular layer doubles in thickness from day 18 to hatching (21 days), with the individual gland becoming a spongy mass of small alveoli in which digestive enzymes are secreted.

More recently, Toner (1965) briefly reported on the electron microscopy of the 7-, 9-, 11- and 18-day embryonic submucosal gland of the proventriculus. He states the following progressions: (1) granular endoplasmic reticulum becomes progressively more organized; (2) mitochondria become larger with increased internal organization; (3) interdigitation of lateral cell membranes increases and slight basal infolding is evident at day 18.

The work herein reported concerns both the histological and ultrastructural characteristics evident in the developing compound submucosal gland in the proventriculus of the chick embryo, with special emphasis placed upon the period between days 13 and 18 of incubation.

METHODS AND PROCEDURES

Embryos of white leghorn chickens (*Gallus domesticus*) were harvested on days

[1]Manuscript received March 18, 1968.

6–15, 18, and 21. Four chicks were maintained until four days posthatching. Proventriculi were excised and subsequently prepared for both paraffin and ultra-thin sectioning. Tissue for paraffin procedures was fixed in alcoholic Bouins solution. Osmium tetroxide at 4°C (Millonig, 1961) was used to fix the tissue for ultra-thin sectioning. Standard hematoxylin and eosin procedure was employed for light microscopy. Sections for electron microscopy were double stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). An RCA–EMU–3G electron microscope was employed for ultrastructure study.

OBSERVATIONS

The submucosal tubuloalveolar gland begins as an invagination in the stratified columnar epithelial lining of the proventriculus (Fig. 1). Throughout the next 7 days, the simple invagination increases in depth and size, invading and slowly reducing the relative size of the submucosa (Figs. 2, 3). During this period the developing gland bifurcates many times, resulting in numerous glandular primordia, the epithelium of which is still stratified columnar (Fig. 4).

By day 14 the lobular nature of the gland is apparent, each lobule consisting of a number of smaller lobes destined to give rise to a secretory unit. The epithelial lining of each lobe is reduced, or is being reduced, to a simple columnar type. Cellular debris fills the lumina of the lobules (Figs. 5, 6).

The presumptive secretory units are well established by day 15, each unit being roughly circular in cross section, with the epithelium of each unit being simple, as opposed to stratified (Fig. 7). The lumen of each lobule is free of debris, but the lumina of the individual secretory units appear to contain debris, although no nuclei can be seen in the debris. The apical portions of the cells appear to be ragged and rather diffuse.

By day 18 the secretory units are definitely circular, with epithelium which is now simple cuboidal. The lumina are clear and the apical portions of each cell appear quite regular (Fig. 8).

At hatching (day 21) and four days posthatching, the lumina of the secretory units appear serrate, due to deep clefts between cells (Figs. 9, 10). The lumina continue to be free of debris.

During the period between days 14 and 18, electron microscopy reveals several interesting phenomena. During day 14, a transition from the stratified columnar epithelium to simple columnar epithelium is evident. This occurs by a sloughing of all cells except the basal cells. Three stages can clearly be identified: (1) full stratified columnar epithelium, nuclei being found at various levels with all cells still intact (Fig. 11); (2) transitional stages with nuclei of the basal cells reverting to a single plane, the lumen containing some debris, while the more apical cells have a loose connection with the basal cells (Fig. 12); (3) simple columnar epithelium, all nuclei having assumed a distinct basal position and all cells being in contact with the basement membrane (Fig. 13).

Further change in the gross structure of the cells is apparent at day 15. The apical portion of the simple columnar epithelium becomes elongated and slender beyond the point of the terminal bar (Fig. 14) and eventually is sloughed from the cell (Fig. 15). The remaining portion of the cell begins to assume the typical shape of a cuboidal cell, with relatively scant cytoplasm and a spheroidal nucleus. By day 18, the established cuboidal nature of the cells forming the presumptive secretory unit is evident (Fig. 16).

Coincident with the changes described, other pertinent ultra-structural changes occur. Between days 7–13, the lateral cell membranes of the stratified columnar epithelium exhibit interdigitation, with the greatest amount by far being seen in the basal cells. By day 14 interdigitation ranges along the entire margin from just distal of the terminal bar to just above the basement membrane in the basal cells (Fig. 12). At day 18, in addition to the interdigitation, desmosomes appear

for the first time in close proximity to the terminal bars. The lateral membrane now exhibits a definite linear pattern of structure: a terminal bar at the point of contact between cells at their apices, followed by a non-interdigitating segment; next a desmosome, followed by profuse interdigitation to the base of the cell (Fig. 16).

DISCUSSION

The fact that some change occurs between days 14–18 in the glandular epithelium in the presumptive secretory unit of the proventriculus has been reported by Hibbard (1942), Dawson and Moyer (1948), Van Alten and Fennell (1957), Romanoff (1960), and Toner (1965). None of these investigators, however, states what actually happens.

The reorganization of the epithelium in the presumptive secretory unit occurs by means of two separate phenomena: (1) sloughing of superficial cells and (2) subsequent remodeling of the remaining cells. Between days 13 and 14 each presumptive secretory unit is composed of a wall of stratified columnar epithelium (Fig. 4). During day 14 a sloughing occurs of all cells except the basal cells, transforming the epithelium to simple columnar.

The functional cell of the definitive secretory unit is not columnar but cuboidal. Therefore one of two events must occur: either (1) new cuboidal cells must be produced by mitosis, or (2) the remaining cells must be remodeled. Figures 14, 15 and 16 indicate that it is the latter process which occurs. The apical portions of the remaining cells bulge well into the lumen and slough off at the level of the terminal bars, thus transforming the columnar cells into cuboidal cells.

This transformation is also apparent under light microscopy, but is by no means as vivid. The lumina of the presumptive secretory units are highly irregular in outline, showing lightly staining debris (Fig. 7). The epithelium of each unit is shown to be simple, as opposed to stratified, but the cells cannot be described as either columnar or cuboidal as a whole, but rather appear to be in a state of flux.

On day 18, the definitive secretory unit exhibits obvious differences (Fig. 8). Here the cells are cuboidal, their apices firm, and the lumina relatively free of debris. Electron micrographs of this tissue at day 18 show the cells to have com-

EXPLANATION OF FIGURES

FIGURE 1. Cross-section of day 6 proventriculus. Arrow indicates invaginating stratified columnar epithelium. ×50.

FIGURE 2. Cross-section of day 8 proventriculus showing deep penetration of mesenchyme by epithelial invaginations. ×50.

FIGURE 3. Cross-section of day 11 proventriculus showing bifurcating primary glands. ×50.

FIGURE 4. Cross-section of day 13 proventriculus showing further development by bifurcating. Arrows indicate points where the stratified nature of the epithelium is very evident. ×50.

FIGURE 5. Cross-section of day 14 proventriculus. Sloughing of supernumerary cells is evidenced by the presence of cellular debris in the gland lumina (arrows). ×50.

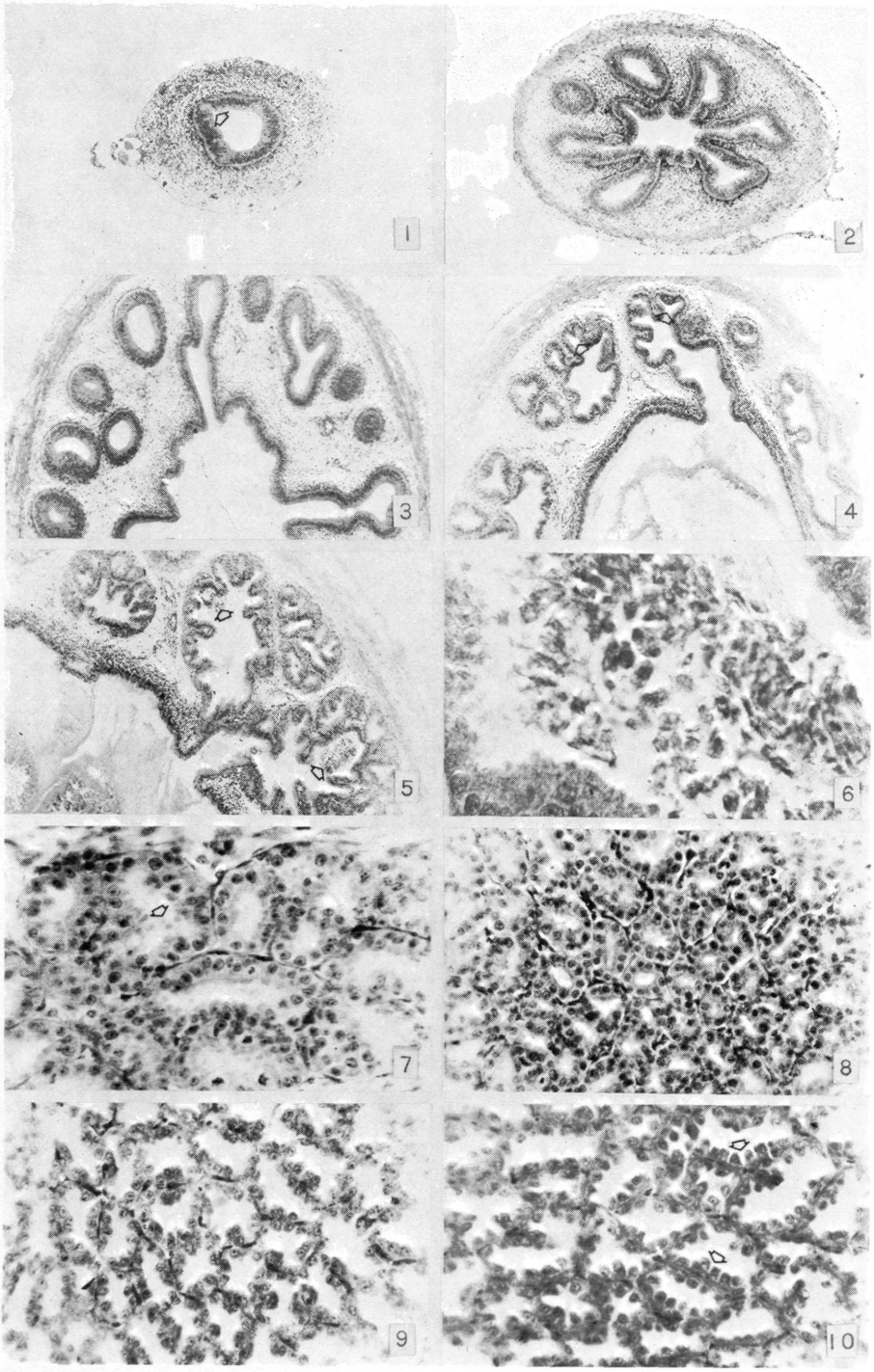
FIGURE 6. Higher magnification of cellular debris shown in Figure 5. Although somewhat deteriorated, nuclei and cytoplasm can be seen in the debris. ×480.

FIGURE 7. Section of presumptive secretory units of day 15 proventriculus. The apical portions of the cells are irregular. The lumina appear to contain debris (arrow). Cells in transitional stage. ×240.

FIGURE 8. Section of presumptive secretory units of day 18 proventriculus. The cell apices appear to be quite stable and the lumina relatively free of debris. Cells are cuboidal. ×240.

FIGURE 9. Section of secretory units of day 21 (hatching) proventriculus showing clefts between cuboidal cells. ×240.

FIGURE 10. Section of secretory units of day 25 (4 days posthatching) proventriculus. Deep clefts (arrows) appear between almost all the cuboidal cells. ×240.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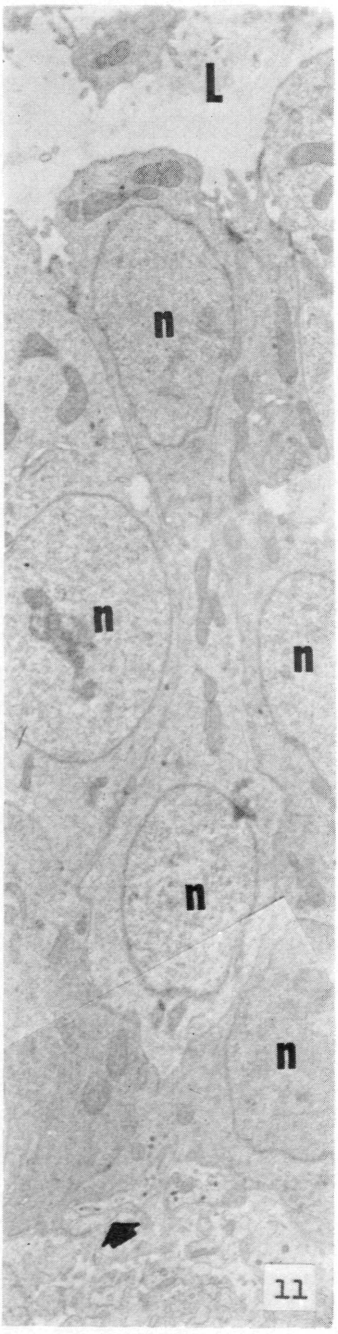

FIGURE 11. Section of entire stratified columnar epithelial wall of presumptive secretory unit of day 14 proventriculus. n, nuclei at different levels; l, lumen of unit; arrow, basement membrane. ×3,300.

pleted the transformation and to have acquired the form of the functional cell (Fig. 16). From this time until after hatching, the cells forming the secretory units exhibit little change.

Conditions in the lateral cell membranes seem to correlate with the transition. Terminal bars and desmosomes are considered to be aids to cellular adhesion. Whether interdigitations do more than increase surface area is questionable. The fact that, prior to stabilization, interdigitation is highly evident only in the basal

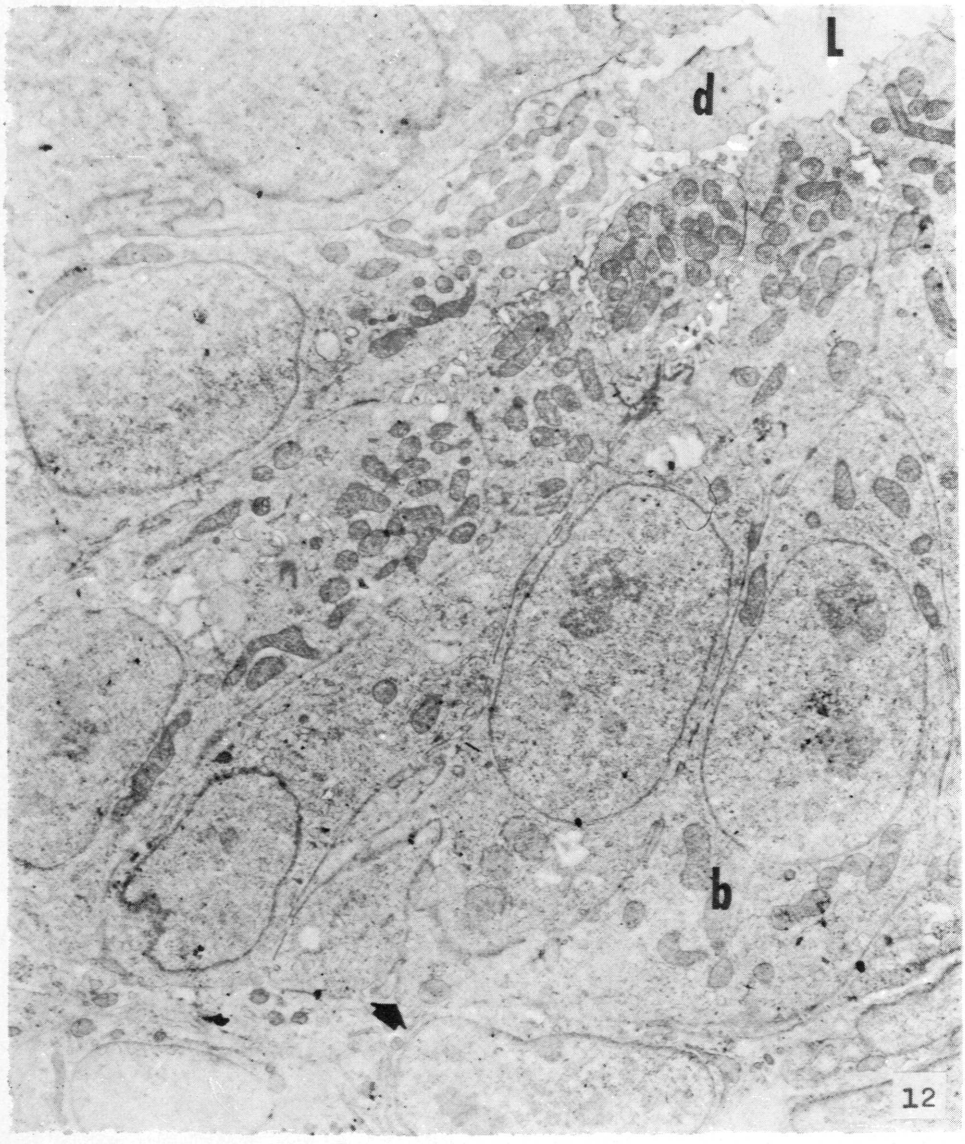

FIGURE 12. Section of stratified columnar epithelial wall of presumptive secretory unit of day 14 proventriculus in transitional stage. d, cellular debris; l, lumen; b, basal cell; arrow, basement membrane. ×5,200.

cells and just slightly in the superficial cells suggests that interdigitation may add to the physical stability of the basal cells, because they remain and all others are lost. As the cells remodel from columnar to cuboidal, the apical portion is sloughed only to the point of the terminal bar, again suggesting a stabilization point. Once the remodeling is completed, desmosomes appear adding to the stabilization of the basal cells.

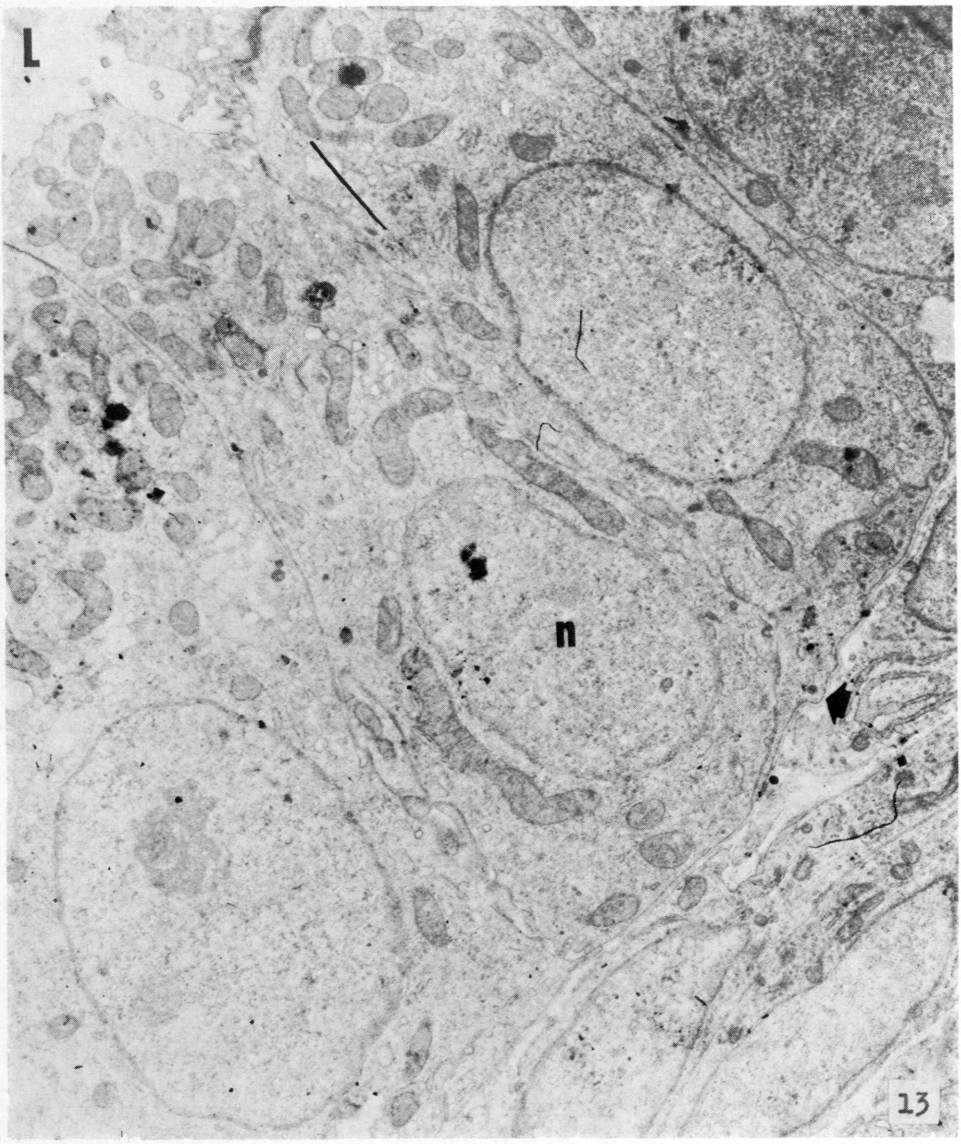

FIGURE 13. Section of epithelial wall of presumptive secretory unit of day 14 proventriculus. Wall composed of simple columnar cells. n, nucleus; l, lumen; arrow, basement membrane. ×7,000.

A progression of cellular attachment is then postulated on the basis of the changing character of the lateral cell membrane. Beginning at day 7, besides the presumed presence of cell cement, terminal bars and slight interdigitations are found. The degree of interdigitation increases, especially in the basal cells, until by day 10 it tends to stabilize. After transition to simple columnar epithelium, the basal cells remodel to columnar cells by sloughing of apical cell portions, the

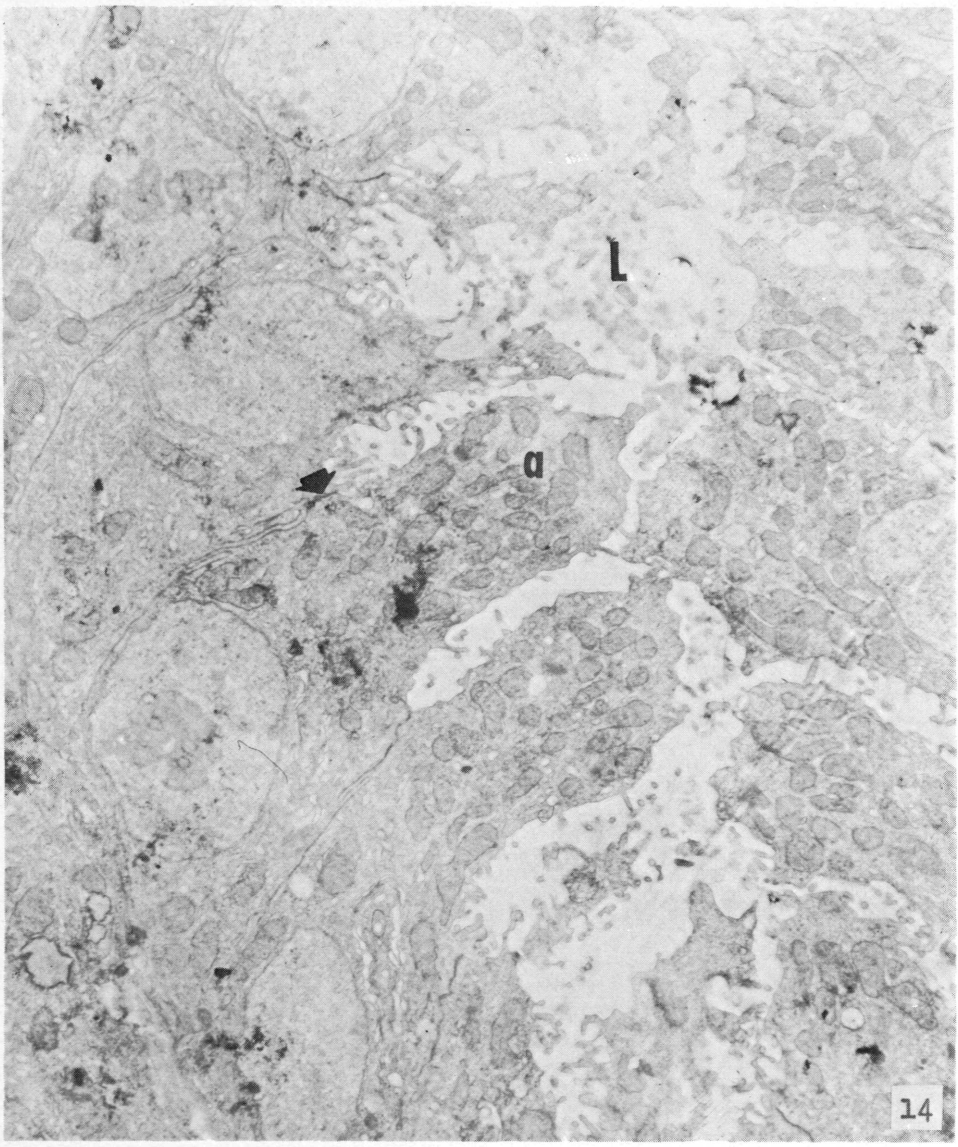

FIGURE 14. Section of presumptive secretory unit of day 15 proventriculus. Apices (a) of columnar cells beyond terminal bar (arrow) are in an elongated state preparatory to being sloughed into lumen (l). ×5,200.

amount of apical loss from these cells being regulated by the position of the terminal bar. Once the presumptive secretory cells reach their definitive shape, desmosomes appear, strengthening the bonds between cells. This condition continues only temporarily, for, after hatching, the pattern of attachment again changes.

A characteristic serrated appearance is evident after hatching. At this time the cells appear to be secretory. Light microscopy shows the cells to be in close

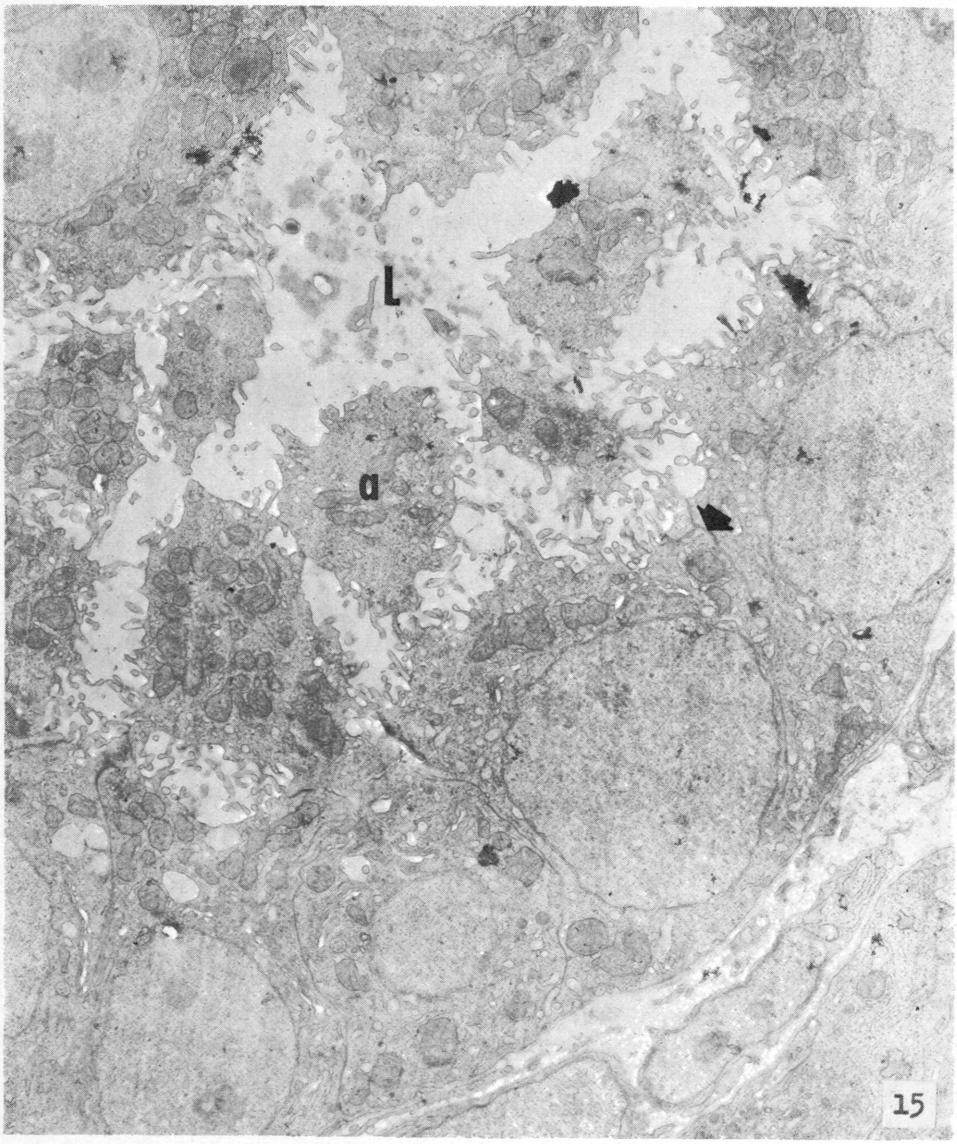

FIGURE 15. Section of presumptive secretory unit of day 15 proventriculus showing sloughing of apical portions (a) of columnar cells into lumen (l). This is the conversion of the columnar to the cuboidal type. ×5,200.

contact at their bases. Electron microscopy indicates the intercellular clefts to be artifactual, probably due to histological preparation, for the entire area between terminal bars and basement membrane is filled with interdigitations, so much so, in fact, that it is difficult to delineate one membrane from another. No desmosomes are apparent at this time, a fact which no doubt helps to account for the separation of cells, thus forming clefts. It is interesting to note that Toner (1963)

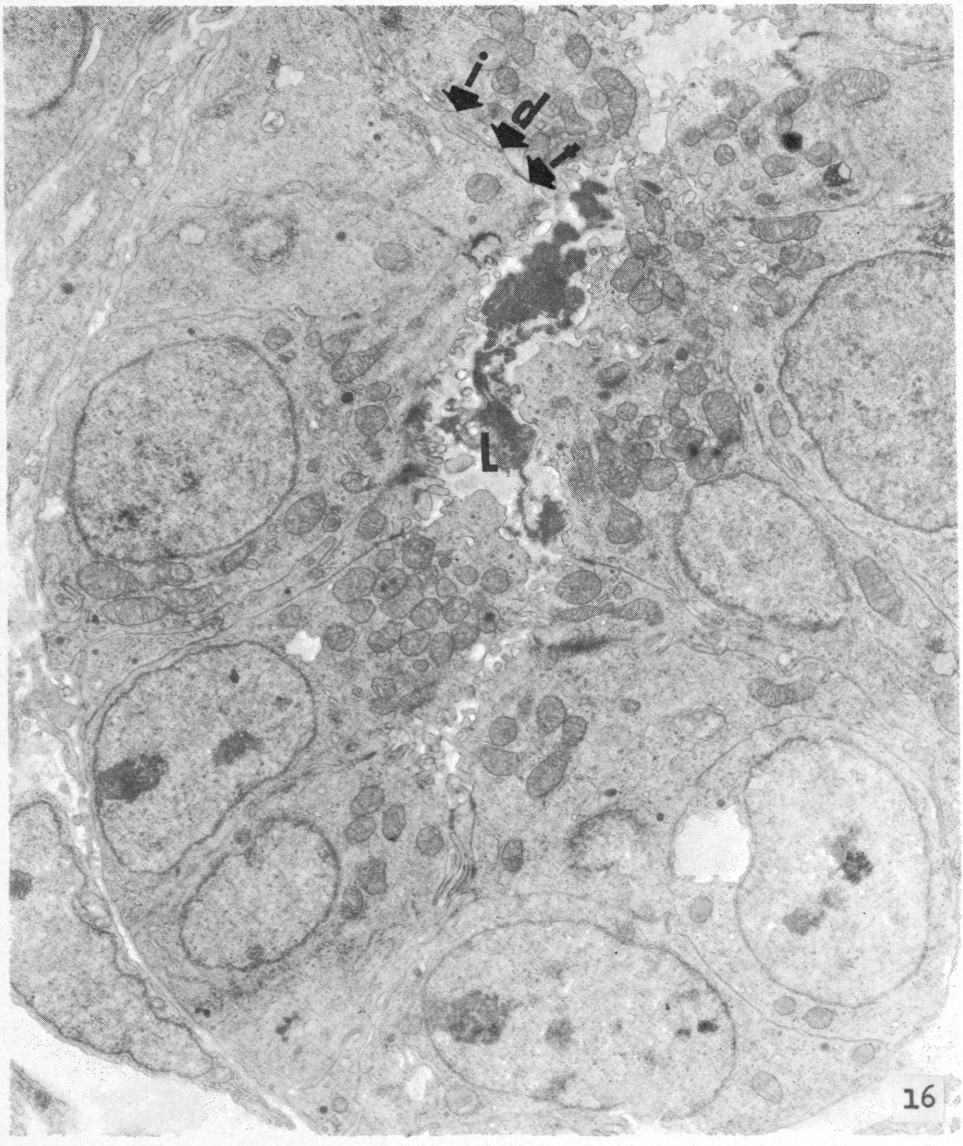

FIGURE 16. Cross-section of presumptive secretory unit of day 18 proventriculus. The cuboidal nature of the cells is evident. The lateral cell membranes exhibit the terminal bar (t)—desmosome (d)—interdigitation (i)—sequence. ×5,200.

reports only slight interdigitation between lateral cell membranes in the adult, analagous to the embryonic state of days 15 and 18. He also states that desmosomes are absent in the adult and makes no mention of them in his work with embryos (Toner 1965).

ACKNOWLEDGMENT

The study was undertaken at The Ohio State University with the support of Science Faculty Fellowship Number 64106, administered by the National Science Foundation. I wish to express my sincere gratitude to Dr. John W. Price and Dr. Wayne B. Parrish for their kind and valued assistance during this study.

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